REMARKS

Claims 2-3, 18-20, 23-30, 33-35, 37, 39-40, 43, 45-51, 53, 55-58, 61, and 65-66 are pending in this application. The examiner found the Applicants' prior explanation over the cited prior art persuasive, but issued an Office Action dated December 11, 2007 (the "Office Action"), in which she (1) rejected claims 49-51, 53, and 55-58 under 35 U.S.C. 112, second paragraph, as being indefinite due to the use of "wave like" notation to show the points of tautomeric change in the ionic compounds in equilibrium as shown on page 6 of the detailed description; (2) rejected claims 45-48 under 35 U.S.C. 102(b) as being anticipated by U.S. Patent No. 6,174,670 to Wittwer, et al. (the "Wittwer Patent") and Nurmi et al. (Anal. Biochem., Vol. 299, pp 211-217, December, 2001)(the "Nurmi Reference"); (3) rejected claims 17-20, 23-24, 39, 41-42 as being anticipated by Higuchi et al. (Biotechnology, Vol. 10, pp. 413-417, 1992) (the "Higuchi Reference"); (3) rejected claims 2-3, 18-19, 23-30, 33-35, 37, 39-43, 45-51, 53, 55-58, and 61 under 35 U.S.C. 102(b) as being anticipated by U.S. Patent No. 5,563,037 to Sutherland et al. (the "Sutherland Patent"); (4) Rejected claims 65-6 under 35 U.S.C. 103(a) as being unpatentable over the Higuchi Reference in view of the Nurmi Reference.

Claims 17 and 41-42 have been cancelled. Claims 18, 20, 23-26, 34, 35, 37, and 39 have been amended. Claim 18 has been rewritten in independent form, including all limitations of its base claim. Claim 24 has been rewritten to include the subject matter of now-cancelled claim 17. Claims 20, 34, and 35 have been amended to change dependency to claim 24, rather than now-cancelled claim 17. Claims 23, 25, 26, 37, and 39 have been amended for consistency with the amendments to claim 24. No new matter has been added by way of these amendments.

In this response, Applicants respectfully traverse the Examiner's rejections discussed above.

I. Claims 49-51, 53, 55-58 are not indefinite.

The examiner found the Applicants' claims 49-51, 53, and 55-58 indefinite under 35 U.S.C. 112, second paragraph, due to the use of "wave like" notation. Applicants respectfully submit that the nature of the bonds suggested by the "wave like" lines is discussed in paragraph 68 of the present application, noting that the bonds and charge at each atom are

tautomeric in nature, and will exist in several forms in equilibrium. Because the notation is defined in the specification such that one of ordinary skill in the art can determine the structure of the molecule described, and one of ordinary skill in the art will appreciate that such a molecule will occur in several tautomeric forms as shown, rather than in a static bond fashion, Applicants submit that the use of the "wave like" bonds are not indefinite, but are defined as disclosed in the specification.

II. The Wittwer Patent Does not anticipate claims 45-48.

Claims 45-48 have been rejected as anticipated by the Wittwer Reference. In particular, the Examiner cites to col. 11, lines 10-38; col. 13, lines 26-67; col. 14, lines 1-29; col. 15, lines 26-45; col. 30, lines 30-33 and lines 61-67; col. 31, lines 1-4; Fig. 15; col. 71, lines 15-67; col. 17, lines 1-46; Fig. 13-26.

Respectfully, many of the cited sections of the Wittwer Reference relate to generation of amplification curves (see in particular col. 11, lines 10-38; col. 15, lines 26-45; col. 30, lines 30-33 and lines 61-67; col. 31, lines 1-4; Fig. 15; col. 17, lines 1-46; and Figs. 14-25. While claims 45-48 require amplification in the presence of a dsDNA binding dye, the claims only require generating a melting curve, not generating an amplification curve. Accordingly, the curves shown and discussed in these cited portions of Wittwer are not relevant to the present claims.

Other sections cited by the Examiner (col. 13, lines 26-67, col. 14, lines 1-29, and col. 71, lines 15-67) relate to melting curves, but none of these sections discuss normalizing the melting curves, and comparing normalized melting curves. Instead, the Wittwer Reference discusses converting the melting curves into melting peaks. This is done by taking the derivative (or negative derivative) of the melting curves (see, e.g., Figs. 41B and 43B of the Wittwer Reference). Inflections in the raw melting curve data are then represented as melting peaks, with each peak corresponding to the Tm of a component of the sample. As shown in 41B, mixed products can be identified in this manner. Melting peak analysis is useful to distinguish amplification products with Tms that vary by a few degrees or more. However, as the Tms approach each other, distinguishing amplicons can be quite difficult. Wittwer does not teach or suggest normalizing the melting curves.

The present claims require normalization of the melting curves. Normalization is described in the present specification in Example 10, page 39, lines 8-14. Normalization is

not required of melting curves when doing melting peak analysis, and normalization was not done on melting curves in the Wittwer Reference. It was not until the present application, with the high resolution melting curves, that one could genotype by comparing the shape of melting curves. This is graphically represented by comparing Fig. 12A (raw melting data) of the present invention to Fig. 12B (normalized melting curves). Because Wittwer does not teach normalization of melting curves and Wittwer certainly does not teach comparing normalized melting curves, applicants respectfully submit that Wittwer does not anticipate claims 45-48.

Furthermore, Wittwer does not disclose plotting the difference between normalized melting curves and temperature shifting melting curves. Wittwer col. 70, line 48 through col. 71, line 14 is a claim relating to determining the completion of an amplification. There is no mention in this claim whatsoever regarding a melting curve. Col. 17 lines 1-5, 29-35, and 47-52 all describe figures that are themselves amplification curves or relate to data obtained from amplification curves. There is no suggestion in this text for temperature shifting or doing difference plots of melting curves. Col. 17 lines 53-63 show raw data from temperature cycling during amplification. Again, there is no suggestion in this text for temperature shifting or doing difference plots of melting curves.

Please note that there is often some confusion between melting peaks and difference plots. While the two can look very similar, they are different mathematical manipulations of melting curves. As discussed above, melting peaks are generated using the derivative (or negative derivative) of the melting curve. Melting peaks provide information on the Tm of the nucleic acids present in the sample. As described on page 40, lines 5-10 of the present specification, difference plots are generated by selecting one melting curve as the standard, with a zero value across all temperatures, and subtracting all other curves from the standard. Subtle differences in shape of the melting curves can then be easily visualized, as seen in Fig. 12D, allowing ready visualization of similar melting curves. While melting peaks were used in Wittwer, there is absolutely no suggestion of the use of difference plots. Applicants believe that melting curve difference plots are a novel.

Applicants respectfully request withdrawal of this rejection.

III. The Nurmi Reference Does not anticipate claims 45-48.

Claims 45-48 have been rejected as anticipated by the Nurmi Reference. In particular, the Examiner cites to page 213, col. 1, paragraph 1; page 214, col. 2, paragraph 1; page 215, col. 1 lines 2-27; and page 215, col. 2, paragraph 2.

Respectfully, the cited sections of the Nurmi Reference relate to amplification curves. There is absolutely no discussion whatsoever in Nurmi regarding melting curves, as required by the present claims. Furthermore, the amplification curves that are compared in Nurmi are generated using a labeled probe, not a dsDNA binding dye. While ethidium bromide is used, this dye is used only as a positive control, to show that amplification has occurred. Nurmi does not teach comparing curves generated from ethidium bromide.

Additionally, Nurmi does not teach normalizing the curves. To do so would undermine the genotyping, which is done by comparing signal strength, as shown in Fig. 2. If the curves were normalized, there would be no way to distinguish the genotypes.

Finally, while Nurmi plots two amplification curves on the same plot in Fig. 2, Nurmi does not temperature shift the curves nor plot the difference between the two curves, as required for claims 46-48.

Since Nurmi does not teach generating melting curves at all, does not teach normalizing the curves, and does not teach comparing curves generated using a dsDNA binding dye, applicants respectfully submit that the Nurmi Reference does not anticipate claims 45-48.

IV. The Higuchi Reference does not anticipate claims 17-20, 23-24, 39, 41-42.

Claims 17-20, 23-24, 39, and 41-42 have been rejected as anticipated by the Higuchi Reference. However, each of these claims depend from independent claim 24, which recites the limitation of "mixing a dsDNA binding dye having a percent saturation of at least 50% with a sample comprising a target nucleic acid and primers . . . ." "Percent saturation" is defined at page 4, lines 8-15 of the present application. For a given dye provided at maximum PCR compatible concentrations, the percent saturation is "the percent fluorescence compared to fluorescence of the same dye at saturating concentrations, i.e., the concentration that provides the highest fluorescence intensity possible in the presence of a predetermined amount of dsDNA." Thus, the percent saturation is a ratio (expressed as a percentage) between the fluorescence at maximal PCR compatible concentration and the fluorescence at the concentration at which the dye reaches its maximal fluorescence intensity.

The Higuchi Reference discloses the use of ethidium bromide ("EtBr") as the binding dye for performing PCR. See, e.g., p. 414, "PCR in the presence of EtBr." In order to assess the affect of EtBr in PCR, amplifications of the human HLA DQα were performed . . . ." There is absolutely no question that ethidium bromide may be used in PCR in appropriate concentrations. However, as shown in Table 1, page 45 of the present disclosure, ethidium bromide has a percent saturation of 12%. As such, the Higuchi Reference teaches the use of a binding dye that inhibits PCR at saturating conditions. Applicants respectfully submit that the Higuchi Reference does not teach a binding dye having a percent saturation of at least 50%. Therefore, Applicants submit that the Higuchi Reference does not anticipate Claim 24 or any of the claims depending therefrom.

V. The Sutherland Patent does not anticipate claims 2-3, 18-19, 23-30, 33-35, 37, 39-43, 45-51, 53, 55-58, and 61.

Claims 2-3, 18-19, 23-30, 33-35, 37, 39-43, 45-51, 53, 55-58, and 61 stand rejected under 35 U.S.C. § 102(b) as being anticipated by the Sutherland Patent.

Claim 24 has been amended to include all of the limitations of claim 17. It is noted that claim 17 has not been included in this rejection. As amended, claim 24 requires amplifying a target nucleic acid and generating a melting curve of the amplified target nucleic acid. Respectfully, Sutherland does not teach melting curves. The sections cited by the examiner all relate to amplification of the sample. In particular, col. 3, lines 40-59 teaches "measure of the presence or amount of the double-stranded target nucleic acid during the amplification step (col. 3, lines 52-54), col. 9, lines 1-28 teaches that "[d]etectable signal can be monitored at any time in the amplification procedure (col. 9, lines 15-16), and col. 15, line 4-67 teaches that fluorescence is measured after each of 0, 5, 15, 20, 25, 30, 40, and 45 cycles (col. 15, lines 43-45). In fact, the Sutherland reference is devoid of any teaching of generating or comparing melting curves. Claims 2-3, 20, 23, 25-30, 33-35, 37-40, 43, 49-51, 53, 55-58, and 61 all depend from amended claim 24. Many of these claims specify further limitations with respect to melting curves. Applicants respectfully request withdrawal of this rejection as it pertains to these claims.

Furthermore, with respect to claim 33, applicants respectfully submit that the capture probe of Sutherland is not labeled with an acceptor dye to accept fluorescent resonance

energy transfer from the dsDNA binding dye. Rather, as taught at col. 16, lines 61-64, the immobilized capture probe is washed and then a leuco dye solution is added.

With respect to claims 49-51, 53, 55-58, and 61, the Examiner cites to col. 3, 1-39, col. [9], lines 40-67, and col. [26], lines 1-54 of Sutherland for a teaching that the dsDNA binding dye is a compound having the claimed formula. While the compounds of the present claims and those of Sutherland are both asymmetric cyanines, applicants respectfully submit that the formulae recited in Sutherland do not anticipate claims 49-51, 53, 55-58, and 61. For example, in the presently claimed compounds, the Q heteroatom contains two nitrogen atoms in the 6-membered ring. In the Sutherland compounds, there is only a single nitrogen atom in the corresponding structure. Accordingly, the presently claimed compounds are different from those of Sutherland.

Claim 18 has been rewritten in independent form, incorporating all of the limitations of its prior base claim, original claim 24. With respect to claims 18-19, the Examiner cites to col. 17, 38-48 for disclosure of the various wavelengths. According to this text, for both dyes tested (YOYO-1 and YO-PRO-1), the excitation maximum is 491nm, which is outside the claimed range of 410-465nm, and the emission maximum at 509, which is outside the claimed range of 450-500nm. These dyes simply do not fit the excitation and emission maxima required for claims 18-19.

Applicants respectfully request withdrawal of this rejection.

VI. The combination of the Higuchi Reference and the Nurmi Reference does not render obvious claims 65-66.

The Examiner cites Higuchi for its teaching of a method of PCR analysis. The Examiner cites Nurmi for its teaching of a PCR analysis wherein the target is the HLA gene. The Examiner finds that it would be prima facie obvious to modify the teachings of Higuchi with the HLA gene as a target.

However, as discussed above, Higuchi does not teach the use of a saturation dye. Nurmi uses a labeled probe and does not use any dsDNA binding. Further, while both references monitor amplification, neither reference teaches generating melting curves, and neither reference, alone or in combination, teaches or suggests comparing melting curves to determine if the first and second nucleic acids have the same sequence.

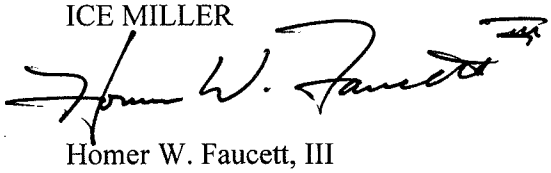
Accordingly, applicants respectfully request withdrawal of this rejection.

Applicants have overcome the examiner's rejections, and respectfully request allowance of the claims for the reasons above. Applicants hereby request for a two month extension of time. Applicants believe that no additional fees are required. In the event Applicants have inadvertently overlooked the need to petition for an extension of time or to pay an additional fee, Applicants conditionally petition therefor, and authorize any fee deficiency to be charged to deposit account 09-0007. When doing so, please reference the above-listed docket number.

If the Examiner has any questions, please contact the undersigned.

Respectfully submitted,

ICE MILLER



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